2014 Vol.9 No.2

JOURNAL OF
OTOLOGY

EFFECTS OF MODERATE NOISE EXPOSURE ON HEARING FUNCTION IN C57BL/6J MICE

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Abstract

Objective To study characteristics of hearing loss after exposure to moderate noise exposure in C57BL/6J mice. **Methods** Male C57BL/6J mice with normal hearing at age of 5-6 weeks were chosen for this study. The mice were randomly selected to be studied immediately after exposure (Group P0), or 1 day (Group P1), 3 days (Group P3), 7 days (Group P7) or 14 days (P14) after exposure. Their before exposure condition served as the normal control. All mice were exposed to a broad-band white noise at 100 dB SPL for 2 hours, ABR thresholds were used to estimate hearing status at each time point. **Results** ABR threshold elevation was seen at every tested frequency at P0 ($P<0.01$). Elevation at high-frequencies (16 kHz and 32 kHz) was greater than at lower frequencies (4 kHz and 8 kHz, $P<0.05$). From P1 to P14, ABR thresholds continuously improved, and there was no significant difference between P14 and before exposure ($P>0.05$). **Conclusion** There is a frequency specific response to 100 dB SPL broad-band white noise in C57BL/6J mice, with the high-frequency being more susceptible. Hearing loss induced by moderate noise exposure appears reversible in C57BL/6J mice.

Key Words: C57BL/6J mice; Noise exposure; Hearing loss; Temporary noise-induced hearing loss; ABR threshold shift

Nowadays, noise pollution has become a public health problem[1-3]. Overexposure to noisy environment can cause various kinds of dysfunction, such as annoyance[4], sleep disturbance[5], cognition impairment[6], hypertension and cardiovascular diseases[7,8], besides hearing impairment [9] which is the primary noise-related dysfunction.

For the auditory system, overexposure to intense sound can cause temporary or permanent noise-induced hearing loss (NIHL) [10,11]. Permanent noise-induced hearing loss is a hot research focus to many scholars. However, compared to long time, high intensity narrow-band noise, exposure to moderate intensity broadband environmental noise of variable time course seems more practically relevant due to the high variable noise sources in modern society. In addition, noises from high-speed railway, automobiles, aircrafts, music and radio players are usually characterized by moderate intensities and broad frequency spectrum.

Researchers have found that temporary NIHL can be associated with changes in microscopic physiological structures in the cochlea. For example, Kujawa and Liberman successfully established a temporary NIHL model using CBA mice which were exposed to an oc-

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tave-band noise of 100 dB sound pressure level (SPL) for 2 hours [12]. The study showed that the threshold elevation at each frequency fully recovered to its pre-exposure level over 2 weeks after overexposure, and that acoustic overexposures could cause acute loss of afferent nerve terminals and delayed degeneration of the cochlear nerve, although cochlear sensory cells were intact [12]. Lin et al subsequently established a temporary NIHL model using guinea pigs and obtained similar results [13]. However, no ideal temporary NIHL model using C57BL/6J mice has been reported until now. This may be due to relatively few studies on C57 mice which tend to show presbycusis early and are seemingly not suitable for long-term studies as a simple NIHL model. While recent studies have showed that hearing at 32 kHz and higher frequencies in C57 mice begins to gradually decline at 3 months after birth, there is no hearing loss at lower frequencies at six months or later [14-16], indicating the feasibility for studies on temporary NIHL in these mice in the first three months. Davis et al once carried out hearing sensitivity studies with the C57 and CBA mice using narrow-band noise of various intensities, and demonstrated appropriate noise conditions which could induce permanent hearing loss (HL) for C57 mice. But when C57 mice were exposed to noises at relatively low intensities, no consistent temporary or permanent threshold shifts could be established [17]. We have successfully constructed a hearing loss animal model of aminoglycoside induced hearing impairment using C57BL/6J mice[18, 19]. Therefore, establishing a NIHL model in C57 mice may benefit future studies on the complex hearing dysfunction in presbycusis and aminoglycoside induced hearing loss. In this study, we chose broad-band white noise as the exposure to study its effect on hearing in C57BL/6J mice.

Materials and methods

Animals

Male C57BL/6J mice (5-6 weeks of age) with normal auditory brainstem response (ABR) thresholds and no middle or inner ear disease were provided by Animal Center of Chinese Military Medical Sciences Academy in this study. The mice were randomly divided into five groups (5-7 mice in each group) to be studied immediately after exposure (P0) or 1 day (P1), 3 days (P3), 7 days (P7) or 14 days (P14) after noise exposure. Their condition before exposure were used as the normal control. All procedures were approved by the Animal Care and Use Committee of PLA General Hospital.

Acoustic Overexposure

All experimental mice were exposed to a broad-band white noise at 100 dB SPL for 2 hours. During the exposure, the animal was kept in a 6cm×6cm×10cm wire cage unrestrained (1 animal/cage). The cage was suspended directly below the horn of the sound-delivery loudspeaker in a small, reverberant chamber. Noise calibration to target SPL was performed immediately before each exposure session with a standard sound level meter. During calibration, the probe of sound level meter was placed inside the cage, and the variation of the sound level was less than 1 dB SPL in various parts of the cage.

ABR Testing

The ABR threshold was acquired pre-exposure and at different post-exposure time points with the TDT hardware and BioSig software (USA). Mice were anesthetized with 10% chloral hydrate (0.0045-0.005 ml/g, i.p.) and kept warm on a heating pad in a soundproof electrically shielded room. Subdermal needle electrodes were inserted at the vertex and ventrolaterally to both ears after anesthesia. The distance between testing earphone and the external ear canal was approximately 0.5 cm. Acoustic stimuli used in our study were clicks and tone bursts (rise/fall time: 1 ms, duration: 4 ms) presented in 5 dB steps descending from 90 dB SPL. Scanning time was 10 ms, and 1024 sweeps were averaged with 300-3000 Hz filtering band-width. At each frequency, threshold was determined as the lowest level at which a repeatable wave III could be obtained,

Preparation of the Organ of Corti

After ABR testing, mice were sacrificed by cervical dislocation. The temporal bone was removed after decapitation, and the cochlea was quickly separated.

Under dissecting microscope, a hole was opened at the apex of the cochlea and the round and oval windows were opened with a needle. The cochlea was carefully perfused with 4% paraformaldehyde (PFA) solution via the apex, then fixed with 4% PFA solution at 4°C overnight. Next, the specimen was decalcified in 10% ethylenediaminetetraacetic acid (EDTA) solution for 3.5 hours. The cochlea shell was removed from apex to base under a dissecting microscope in 0.01 mmol/L PBS solution. The basilar membrane was separated, and the vestibular membrane and tectorial membrane were removed.

Immunofluorescence Staining

The isolated specimens were put into PBS and washed thrice (5 mins per time), and then pre-incubated for 30 mins at room temperature in PBS blocking solution with 5 % normal goat serum. Next, the samples were washed in PBS three times (5 mins per time) and incubated with tetramethyl rhodamine isothiocyanate labeled Phalloidin (TRITC-Phalloidin, 1: 200) at 37℃ away from light for 30 mins. After incubation, the samples were washed in PBS thrice (5 mins per time) and stained with DAPI solution (4', 6-diamidino-2-phenylindole, Santa Cruz) away from light for 15 mins, then washed twice (5 mins per time). The basement membranes were spreaded on the slide and mounted with glycerol under a dissecting microscope, and temporarily preserved in a dark wet box placed in a refrigerator at 4℃.

Confocal Microscopy Imaging

The specimen was examined using an Olympus confocal microscopy (Japan) with a ×60 oil immersion objective lens. Excitation wavelength was 408 nm and 594 nm, and local digit was magnified two times. The sequence scan was conducted successively from top to bottom and the scanning interval was set to 0.2 μm in this study.

Statistical Analysis

In order to reduce the effects of experimental errors and inter-individual differences, self-control and paired design were chosen. The ABR thresholds before and after noise exposure within each mouse were used as a data pair for comparison. ABR threshold shifts were expressed as mean ± SD. All data were analyzed using the SPSS 19.0 statistical software. Pre- and post-noise exposure ABR thresholds at different time points were compared using the Paired-sample T test. ABR threshold shifts at each frequency at P0 were compared using the one way ANOVA Student-Newman-Keuls test. P values<0.05 were considered statistically significant.

Results

Dynamic changes of ABR thresholds after noise exposure

Compared with pre-exposure levels, ABR thresholds showed dramatic elevations at P0 for all tested frequencies and were greater than all other time points ($P<0.01$): 11.92 ± 7.51 dB for clicks, 11.92 ± 5.60 dB at 4 kHz, 12.31 ± 5.99dB at 8 kHz, 21.54 ± 8.75 dB at 16 kHz, and 20.00 ± 8.90 dB at 32 kHz. At P1, ABR threshold shifts began to decrease although still significantly elevated compared with pre-exposure ($P< 0.01$). At P3, ABR thresholds at all frequencies showed gradual recovery, although still elevated compared to pre-exposure levels ($P<0.01$ at 4, 8 and 16 kHz and $P< 0.05$ at 32 kHz and for clicks). At P7, ABR thresholds continued to show improvement, with thresholds approaching pre-exposure levels at 32 kHz and for clicks ($P>0.05$). Still, ABR thresholds remained elevated compared to pre-exposure at 4 kHz ($P<0.01$) and at 8 kHz and 16 kHz ($P<0.05$). At P14, thresholds at all frequencies had fully recovered to their pre-exposure levels ($P>0.05$), as shown in Table 1.

Frequency characteristics of moderate noise expo-

Table 1. ABR threshold Changes after exposure to 100 dB SPL broad-band white noise

Time points	ABR Threshold Shifts(dB)				
	Click	4kHz	8kHz	16kHz	32kHz
P0 (n=13 ears)	11.92±7.51 △	11.92±5.60 △	12.31±5.99 △	21.54±8.75 △	20.00±8.90 △
P1 (n=10 ears)	10.00±9.72 △	9.00±2.11 △	9.50±4.97 △	13.50±4.12 △	15.00±7.07 △
P3 (n=10 ears)	5.00±5.27 *	8.00±3.50 △	8.00±4.22 △	8.00±4.22 △	7.00±4.83 *
P7 (n=8 ears)	4.38±6.23	7.50±3.78 △	6.88±3.72 *	6.25±6.41 *	5.63±9.80
P14 (n=9 ears)	1.11±6.01	1.11±3.33	1.11±4.17	1.67±4.33	2.78±4.41

sure induced hearing loss

As shown in Table 1, ABR threshold shifts were the greatest immediately after exposure to 100 dB SPL white noise for 2 hours, followed by a gradual recovery. One way ANOVA Student-Newman-Keuls test showed that ABR thresholds shifts at high-frequencies (16 kHz and 32 kHz) were greater than at relatively lower frequencies (clicks, 4 kHz and 8 kHz) ($P<0.05$).

Noise-induced hearing loss without morphological changes of hair cells

Inner and outer hair cell nuclei, labeled with DAPI, showed normal arrangement, with no deletion or displacement in all groups (Figure 1). Compared with the control, stereocilia in the middle turn (labeled with TRITC-Phalloidin) showed normal arrangements and morphology, seemingly not affected by the noise exposure (Figure 2).

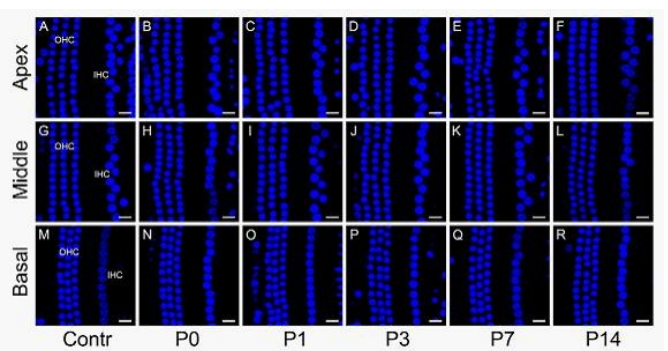

Figure 1. Morphology of cochlear hair cells after exposure to 100 dB SPL broad-band white noise for 2 hours in C57 BL /6J mice. A-F: inner and outer hair cells in the apical turn for the control, P0, P1, P3, P7 and P14 respectively. G-L: inner and outer hair cells in the middle turn at each time point. M-R: inner and outer hair cells in the basal turn at each time point. Confocal microscopy images indicate normal morphology for the control and at each time point, showing one row of IHCs and three rows of OHCs. Blue labeling indicates DAPI-positive hair cell nuclei. Scale bar = 10 μm

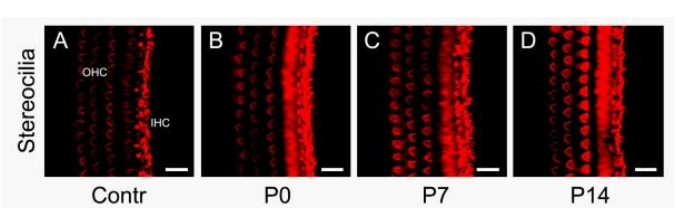

Figure 2. Stereocilia on inner and outer hair cells in the middle turn before (A) and at P0, P7 and P14 (B, C and D) after continuous exposure to 100 dB SPL broad-band white noise for 2 hours, showing unaffected morphology by the noise exposure. Red labeling indicates the stereocilia of HCs. Scale bar = 10 μm

Discussion 3.1 Temporary NIHL affecting high-frequencies

Our data indicate that high-frequency hearing is more susceptible to moderate intensity broad-band white noise exposure. Maximum ABR threshold shifts of each frequency appeared immediately following exposure for all frequencies, especially so for high-frequencies (i.e. 16 kHz and 32 kHz at about 20 dB). After 24 hours, ABR thresholds already showed signs of recovery, which continued over the time course of 2 weeks toward a complete recovery to pre-exposure levels. This is consistent with some reports overseas [12,20]. The maximal ABR threshold shifts were in the range of 10-20 dB over the entire frequency range in our study, while Kujawa and Liberman reported maximal threshold impairment reaching 40 dB at 32 kHz seen at 1 day after exposure with only about 5 dB thresholds at 8 kHz and below. This apparent discrepancy may be caused by different species of mice use, although studies overseas have generally assumed that C57 mice are more sensitive to noise than CBA mice under the same condition [11,17]. The type of noise exposure may also help explain this discrepancy. Kujawa and Liberman used an octave band of noise between 8 kHz and 16 kHz, while broad-band white noise was for exposure in this study. With the same noise intensity and exposure duration, the energy of their octave band noise was mainly concentrated in a limited frequency range between 8 kHz and 16 kHz, while energy of our broad-band white noise was evenly distributed across all frequencies, which may explain why hearing loss in our study was less severe over high-frequencies but more severe over low-frequencies than CBA mice.

Moderate noise exposure and more sensitive structures in cochlea

The current study indicate that the morphology of cochlear hair cells in mice may not be affected by the moderate noise exposure. Overexposure to intense noise can cause permanent hearing loss, often accompanied with acute apoptosis of outer hair cells (OHCs) and degeneration of their affiliated stereocilia [11,21]. These significant pathological changes do not necessarily mean that OHCs and their affiliated stereocilia are the most sensitive components to noise in the cochlea. There were no damage to inner or outer hair cells, while irreversible changes of the afferent nerve endings of spiral ganglion neurons (SGNs) and cochlear nerve took place in Kujawa and Liberman's study[12]. Our study showed similar results. The broad band noise exposure in this study caused no visible changes of the hair cells (HCs). We therefore believe that the hearing loss induced by moderate intensity

broad band noise exposure may not be the results of morphological changes of hair cells. Similarly, in a previous study by the authors on gentamicin induced deafness, treatment with a low dose of gentamicin in C57BL/6J mice revealed that cochlear inner hair cell ribbon synapses were the primary target of ototoxic aminoglycoside stimuli[22]. Therefore, an animal model of temporary NIHL by relatively moderate noise exposure may be ideal for determining the most sensitive structure in the cochlea in the future.

In this study, we successfully created a model of temporary NIHL by a relatively mild noise exposure and demonstrated relevant characteristics of hearing impairment in C57BL/6J mice. This helps lay a foundation for future studies on morphology and pathological mechanisms in hearing loss. Weakness in our study include lack of evaluation of inner and outer hair cells and cochlear inner hair cell ribbon synapses functions, as well as function of the auditory nerve. Additional studies are needed to address these issues.

Acknowledgments

This work was supported by grants from the Liaoning Science and Technology Project (No.2011225017, 2012225021), the National Basic Research Program of China (973 Program) (2012CB967900, 2012CB967901). Additionally, this work was also supported by the grants from the Beijing Natural Science Foundation (5122040), the China Postdoctoral Science Foundation (201003779, 20100470103), and the National Natural Science Foundation of China (NSFC, 31040038).

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(Received June 23, 2014)